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FIELD OF THE INVENTION

Between lines 8 and 9, please insert the following heading:

RECEIVED

OCT-2-4-2001

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DISCUSSION OF THE BACKGROUND

TECH CENTER 1600/2900

Page 5, between lines 7 and 8, please insert the following heading:

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SUMMARY OF THE INVENTION

Page 6, please replace the tables with the following new tables:

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Primers	Nucleotide sequences	Hybridization temperatures	Isoforms amplified
G.257	5'-GGAAGAGGAGACACGGAACA (SEQ ID NO:1)	61	G1, G2, G3
G3.U	5'-GGCTGGTCTCTGCACAAAGAGA (SEQ ID NO:2)		G4, G5, G6
G.526	5'-CCAATGTGGCTGAACAAAGG (SEQ ID NO:3)	61	G1, G4, G5
G3.U	5'-GGCTGGTCTCTGCACAAAGAGA (SEQ ID NO:4)		
G.-3-4	5'-ACCAGAGCGAGGCCAAGCAG (SEQ ID NO:5)	65	G3
G.3.U	5'-GGCTGGTCTCTGCACAAAGAGA (SEQ ID NO:6)		
G.-3	5'-ACCAGAGCGAGGCCAACCCC (SEQ ID NO:7)	65	G2, G6
G3.U	5'-GGCTGGTCTCTGCACAAAGAGA (SEQ ID NO:8)		
G.-3	5'-ACCAGAGCGAGGCCAACCCC (SEQ ID NO:9)	61	G6
G.i4b	5'-AAAGGAGGTGAAGGTGAGGG (SEQ ID NO:10)		
G.526	5'-CCAATGTGGCTGAACAAAGG (SEQ ID NO:11)	61	G5

G.i4b	5'-AAAGGAGGTGAAGGTGAGGG (SEQ ID NO:12)		
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Probes	Nucleotide sequences	Hybridization temperatures (°C)	Isoforms
GR	5'-GGTCTGCAGGTTTCATTCTGTC (SEQ ID NO:13)	60	HLA-G1, G2, G3, G4, G5, G6
G.647 F	5'-CCACCACCCTGTCTTTGACT (SEQ ID NO:14)	60	HLA-G1, G2, G5, G6
G.I4 F	GAGGCATCATGTCTGTTAGG (SEQ ID NO:15)	55	HLA-G5, G6
G.927 F	5'-ATCATGGGTATCGTTGCTGG (SEQ ID NO:16)	55	HLA-G1, G2, G3, G4, G5 and G6

Page 6, before prenumbered line 4, insert the following text:

#### BRIEF DESCRIPTION OF THE DRAWINGS

-Figure 1 illustrates:

(A): the RT-PCR analysis of the HLA-G isoform mRNAs in melanoma cells. pan-HLA-G primers [primer G.257 (exon 2) and 3G.U (untranslated 3' end)] are used for the PCR amplification of the HLA-G transcripts corresponding to the various known HLA-G isoforms. The cDNA from JEG-3 choriocarcinoma cells and first trimester trophoblasts (TRO), and peripheral blood mononucleated cells (PBMC) were used, these cells being used as control cells for high transcription levels and basal transcription levels of HLA-G, respectively. IgR, M8, DRAN and M74 correspond to the amplification of the cDNA of melanoma cell lines. The specific HLA-G bands are revealed by hybridization with the GR-specific probe, which is located on exon 2. The bands corresponding to the transcripts HLA-G1, G2, G3, G4 and G5 are indicated with arrows. The PCR products which were

coamplified during the same reaction using  $\beta$ -actin primers are detected on the same membrane with the aid of a  $\beta$ -actin probe;

(B): this figure corresponds to the RT-PCR detection of alternative transcripts in melanoma cells. Primer 3 is specific for the HLA-G2 and soluble HLA-G2 (G6) isoforms which do not possess exon 3. Primer 3.4 makes it possible to distinguish the HLA-G3 mRNA transcripts. Primers G.526 and 14b amplify specifically the HLA-G5 transcript, which corresponds to the soluble form. The PCR products which were coamplified during the same reaction using  $\beta$ -actin primers are detected on the same membrane with the aid of a  $\beta$ -actin probe;

(C): this figure corresponds to the RT-PCR analysis of the HLA-G mRNA in melanoma cells. pan-HLA-G primers [primer G.257 (exon 2) and 3G.U (untranslated 3' end)] are used for the PCR amplification of the HLA-G transcripts corresponding to the various known HLA-G isoforms. The cDNA from JEG-3 choriocarcinoma cells was used, these cells being used as control cells for high transcription levels. IgR, M8 and DRAN correspond to the amplification of the cDNA melanoma cell lines. The specific HLA-G bands are revealed by hybridization with the GR-specific probe, which is located on exon 2. The bands corresponding to the transcripts HLA-G1, G2, G3, G4 and G5 are indicated with arrows. The PCR products which were coamplified during the same reaction using  $\beta$ -actin primers are detected on the same membrane with the aid of a  $\beta$ -actin probe.

- Figure 2 illustrates the RT-PCR analysis of the HLA-G isoform mRNAs in the biopsies of melanoma metastases (in vivo and ex vivo analysis of skin). The pan-HLA-G primers G.257 and 3G.U are used for the RT-PCR amplification of the HLA-G transcripts from skin metastases *ex vivo* (MEL) and from biopsies of healthy skin from the same patient (HS); JEG-3 cells and first trimester trophoblasts are used as controls (high level of HLA-G

transcription). The HLA-G specific bands are revealed by hybridization with a GR-specific probe which is located in exon 2. The bands corresponding to the transcripts HLA-G1, G2, G3, G4 and G5 are indicated with arrows.

- Figure 3 illustrates the detection of the HLA-G1 proteins in JEG-3 cells but not in IGR and M8 I melanoma cells, with the aid of the monoclonal antibody W6/32: the biotinylated surface proteins of melanoma and JEG-3 cells are immunoprecipitated using the monoclonal antibody W6/32; the immunoprecipitates are separated by SDS-PAGE at 12% and transferred onto cellulose membrane. The class I surface molecules are detected with streptavidin-conjugated peroxidase.

- Figure 4 illustrates the immunoprecipitation of the HLA-G isoforms of IGR melanoma cells with an antibody directed against the heavy chain of free HLA-G and with the monoclonal antibodies 4H84 and HCA2. The cells are labelled for 30 min and immunoprecipitated with the specific antibodies, and the immunoprecipitates are analysed by SDS-PAGE at 10%. The antibody 4H84, which reacts with the HLA-G heavy chain (39-KDa band in JEG-3 cells), exhibits cross-reactions with the HLA-A, -B and/or -C heavy chains (45-KDa band in all the cells tested).

- Figure 5 illustrates:

(A): the effect of HLA-G expression in the IGR melanoma on sensitivity to lysis by the clone YT2C2-PR. K562 cells which are transfected either with the vector alone, or with the HLA-G1 vector containing the cDNA, or the HLA-G2 vector and the M8, M74, IGR and DRAN lines are used as target cells (T). The clone YT2C2-PR is used as an effector cell (E) in an effector cell/target cell (E/T) ratio of 50/1. The results are expressed as the percentage of lysis recorded in 4 h in a chromium 51-release assay. Spontaneous release never exceeds

10% of the maximum release. This experiment is carried out at least 5 times and, each time, produces the same results;

(B): the inhibition of the lysis induced by the clone YT2C2-PR is due to an "off" signal which is transmitted by the IGR and DRAN cells. The M8 line is used as a target cell (T) and is chromium labelled. Clone YT2CT-PR is used as an effector cell (E) in an E/T ratio of 50:1. IGR and DRAM cells are added as inhibitor cells in an inhibitor cell/target cell ratio of 100, 50 and 25:1. 0 indicates that no IGR cell was added in the assay;

(C): the inhibition of the lysis induced by HLA-G-positive melanoma cells (target cells T). This figure illustrates more particularly the effect of HLA-G expression by IGR and DRAN melanoma cells on sensitivity to lysis by the clone YT2C2-PR. Several cell lines which are B-EBV, HLA-G negative [HOM (A3, B27, Cw1), BM (A29, B61 Cw2), SPO (A3, B7, Cw7), SWE (A2, B44, Cw5)] are lysed by the clone YT2C2-PR. This clone is used as an effector cell (E) in an E/T ratio of 50/1. The results are expressed as the percentage of lysis recorded in 4 h in a chromium 51-release assay. Spontaneous release never exceeds 10% of the maximum release;

(D) and (E): these figures show that the M8 HLA-G-negative tumour cells which are transfected with the cDNAs encoding the molecules G1, G2, G3 and G4 inhibit NK lysis (Figure 5E) and the cytotoxic T responses (Figure 5D). Figure 5D comprises, on the x-axis, the effector cells (E) (restricted HLA-A2 lines specific for an influenza peptide)/target cells (T) (transfected M8 lines) ratios and, on the y-axis, the percentage of specific lysis. The table below corresponds to the values obtained in this figure.

E/T ratio	M8-RSV	G1	G2	G3	G4	Genomic
15/1	55%	8%	39%	12%	17%	30%
7/1	52%	6%	42%	10%	14%	25%

3/1	29%	2%	30%	6%	12%	23%
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- Figure 5E comprises, on the x-axis, the effector cells (E) (clone YT2C2-PR)/target cells (T) (transfected M8 lines) ratios and, on the y-axis, the percentage of-specific lysis.

- Figure 6 illustrates the detection of HLA-G transcripts in biopsies of human melanomas. The RT-PCR amplifications are carried out, using the abovementioned primers G.257 and G.3U, on biopsies of healthy skin (HS) and on healthy lymph nodes (HLN), on the one hand, and biopsies of lymph node metastases (LNM1 and LMN2). JEG-3 choriocarcinoma cells are used as control cells for high transcription levels. Specific HLA-G bands are revealed by hybridization with the GR-specific probe which is located on exon 2. The bands corresponding to the transcripts HLA-G1, G2, G3, G4 and G5 are indicated with arrows. The PCR products which were coamplified during the same reaction using the (3-actin primers are detected on the same membrane with the aid of  $\beta$ -actin probe.

- Figure 7 illustrates the RT-PCR analysis of the HLA-G transcripts in the biopsies of primary melanoma tumours and in the derived MPP5 primary cell cultures (*ex vivo* analysis). The abovementioned pan-HLA-G primers are used for the amplification from biopsies of healthy skin (HS1), from skin primary tumours (SPT1) and from tumours in regression (R1) which are obtained from the same patient, and from derived primary cells obtained from a skin tumour tissue (MPP5). The MPP5 cells and the SPT1 biopsy exhibit similar HLA-G transcription levels. JEG-3 cells are used as controls for high levels of HLA-G transcription. The HLA-G-specific bands are revealed by hybridization with a GR-specific probe which is located in exon 2. The bands corresponding to the transcripts HLA-G1, G2, G3, G4 and G5 are indicated with arrows. The PCR products which were coamplified during the same

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reaction using the  $\beta$ -actin primers are detected on the same membrane with the aid of a  $\beta$ -actin-specific-probe.

- Figure 8 illustrates:

(A) the specific detection of HLA-G5 transcripts by RT-PCR in biopsies of melanomas. The amplification of the HLA-G5 transcript from healthy lymph nodes (HLN), from a skin primary tumour (SPT1) and from two biopsies of lymph node metastases (LNM1 and LNM2) is carried out with the aid of the primers G.526 and G.i4b. The band corresponding to the HLA-G5 transcript is detected by hybridization with an I4F probe which is located in intron 4; JEG-3 cells are used as controls (high levels of HLA-G5 transcription). The band corresponding to the HLA-G5 transcript is indicated with arrows. The PCR products which were coamplified in the same reaction using the  $\beta$ -actin primers are- detected on the same membrane with a  $\beta$ -actin-specific probe;

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(B) the immunohistochemical analysis of the soluble HLA-G expression in the LNM1 biopsy. Frozen and acetone-fixed sections of the LNM1 biopsy are positively stained with the anti-melanoma antibody HMB45 (DAKO) and the anti-soluble HLA-G antibody 16G1, whereas the negative control gives no staining, using the Envision anti-mouse, peroxidase system (DAKO) and AEC as substrate.

#### DETAILED DESCRIPTION OF THE INVENTION

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Please delete, in its entirety, the text beginning at page 11, line 30, through page 16, line 28.

Please replace the paragraph bridging pages 18 and 19 as follows:

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The specific HLA-G probes are as follows:

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- GR, specific for exon 2,

- G.647 F (5'-CCACCACCCTGTCTTTGACT (SEQ ID NO:17): specific for exon 4),
- G.I4 F (GAGGCATCATGTCTGTTAGG (SEQ ID NO:18): specific for intron 4),
- and
- G.927 F (5'-ATCATGGGTATCGTTGCTGG (SEQ ID NO:19): specific for exon 5).

Page 19, line 5-12, please replace the paragraph with the following:

The other probes are as follows:

- HLA-A-specific probe

(5'GGAGGACCAGACCCAGGACACG) SEQ ID NO:20),

- HLA-B-specific probe

(5'AGCTCCGATGACCACAACCTGC) (SEQ ID NO:21)

- HLA-C-specific probe (5'TGTCCTAGCTGCCTAGGAG) (SEQ ID NO:22) and

- HLA-DRA-specific probe (TGTGATCATCCAGGCCGAG) (SEQ ID NO:23).

NE Page 31 (Abstract), after the last line, beginning on a new page, please insert the attached Sequence Listing.

### IN THE CLAIMS

Please amend the claims as shown in the attached-marked up copy to read as follows:

2. (Amended) A method for establishing the HLA-G expression profile of a solid tumour with a view to selecting a treatment which is suited to said tumour or with a view to monitoring the evolution of said tumour, comprising:

- (i) removing a tumour sample,
- (ii) preparing a histological section from said sample,